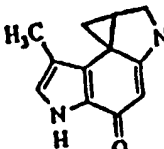


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(21) International Application Number: PCT/US96/00727 (22) International Filing Date: 31 January 1996 (31.01.96) (30) Priority Data: 08/381,355 31 January 1995 (31.01.95) US (71) Applicant (for all designated States except US): SYNPHAR LABORATORIES, INC. [CA/CA]; Taiho Alberta Center #2, 4290-91A Street, Edmonton, Alberta T6E 5V2 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): LOWN, William, J. [CA/CA]; 4704-117A Street, Edmonton, Alberta T6H 3S1 (CA). WANG; Yuqiang [CA/US]; 278 Monroe Drive #17, Mountain View, CA 94040 (US). LUO, Weide [CA/CA]; Apt. 209, 4516 Valiant Drive, N.W. Calgary, Alberta T3A 0Y1 (CA). (74) Agents: MURRAY, Robert, B. et al.; Nikaido, Marmelstein, Murray & Oram, Metropolitan Square, Suite 330 - G Street Lobby, 655 Fifteenth Street, NW, Washington, DC 20005- 5701 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.
(54) Title: CYCLOPROPYLPYRROLOINDOLE-OLIGOPEPTIDE ANTICANCER AGENTS		
(57) Abstract The invention is directed to novel cyclopropylpyrroloindole-oligopeptide compounds which are useful as anti-cancer agents. The novel cyclopropylpyrroloindole-oligopeptide compounds have general structure <div style="text-align: center;">  $\text{H}_3\text{C} \quad \text{N} \text{---} \text{CO} \text{---} \text{R} \text{---} (\text{Het}^1\text{NHCO})_n \text{---} (\text{Het}^2\text{NHCO})_m \text{---} \text{A}, \quad (I)$ </div> (I), wherein Het¹ and Het² are individually selected from the group consisting of pyrrole, imidazole, triazole, thiophene, furan, thiazole, oxazole and pyrazole, R is selected from the group consisting of a valence bond; a C₁-C₆ alkyl; a C₂-C₆ alkenyl; a C₂-C₆ alkynyl; and an ortho, meta or para linked aromatic group, A is selected from the group consisting of a C₁-C₆ alkyl group; and amidine or derivative thereof; a guanidine; a secondary, tertiary or quaternary ammonium salt; and a sulfonium salt, n is 0 to 3, and m is 0 to 3.		

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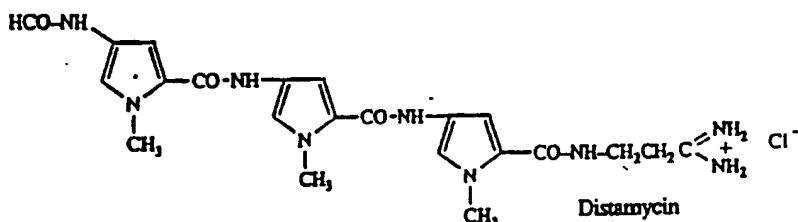
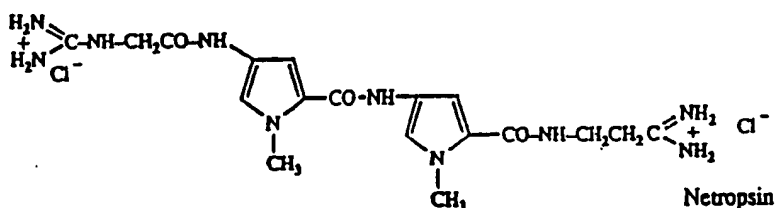
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Cyclopropylpyrroloindole-Oligopeptide Anticancer AgentsField of the Invention

This invention relates to novel cyclopropylpyrroloindole-oligopeptides which are useful as anticancer agents.

Background of the Invention

The title compounds are related to the family of natural oligopeptide antiviral antitumor antibiotics which include netropsin (Julia, M., Preau-Joseph, N.C.R., *Hebd-Seances, Acad. Sci.*, 1963, 257, 1115) and distamycin (Arcamone, F., Orezzi, P.G., Barbier, W., Nicoletta, V., Penco, S., *Gazz. Chim. Ital.*, 1967, 97, 1097).

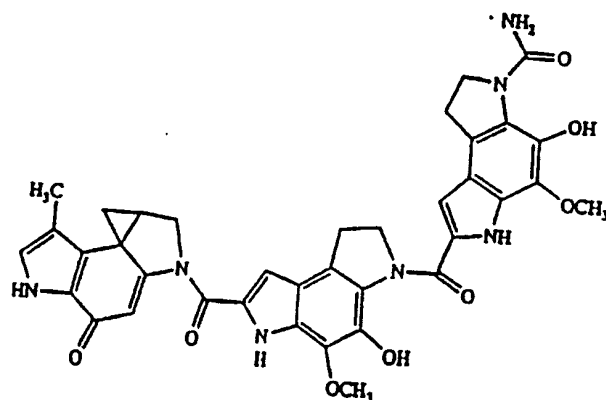


These structures contain pyrrole moieties connected by peptide bonds and with side chains, at least one of which is positively charged, i.e. an amidine group, N-formyl or a group of the guanidyl type.

Of this group of natural oligopeptide antibiotics only distamycin has been used as a therapeutic agent under the name of Stallimycin Hydrochloride in the form of a 1% cream, ointment or paste (Martindale, *The Extra Pharmacopoeia*, Twenty-eighth Edition, p. 825, 1982) in treatments of infections produced by herpes simplex,

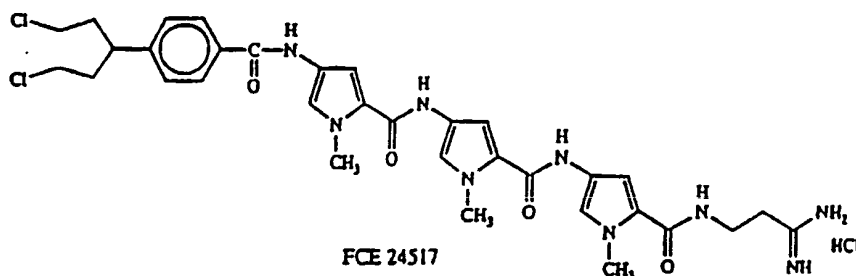
herpes zoster and vaccinia viruses. The external use limitation of distamycin is due to its high cytotoxicity and a low therapeutic index which in the case of herpes viruses is about 3.

- 5 Other pertinent compounds are the natural product CC-1065 (Reynolds, V.L., Molineux, T.J., Kaplan, D., Swenson, D.H., Hurley, L.H., *Biochemistry*, 1985, 24, 6228), and the synthetic agent



CC-1065

- 10 FCE 24517 (Arcamone, F.M., Animati, F., Barbieri, B., Configliacchi, E., D'Alessio, R., Geroni, C., Giuliani, F.C., Lazzari, E., Menozzi, M., Mongetti, N., Penco, S., Verini, A., *J. Med. Chem.*, 1989, 32, 774.



FCE 24517

Whereas CC-1065 itself is too toxic for clinical applications because of the delayed death syndrome three synthetic analogs produced by Upjohn are in clinical trials. Compound FCE 24517 is also undergoing clinical trials at this time.

Summary of the Invention

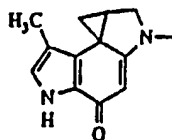
The title agents are compounds of the following general structure:

10



where

CPI=



R = CH₂ or CH₂CH₂ or CH=CH (Z or E), or C≡C; n = 0 to 3, m = 0 to 3, A is a moiety consisting of either an alkyl group, CH₃, CH₃CH₂, CH₃CH₂CH₂ or CH₃CH₂CH₂CH₂, or a group bearing a positive charge, e.g. amidine and derivatives, secondary, tertiary or quaternary ammonium salts, and sulfonium salts, Het is a monocyclic heterocyclic moiety, e.g. pyrrole, imidazole, triazoles, pyrazoles, thiazole, thiophene, furan, and oxazole. Zusammen (Z) and Entgegen (E) are recognized abbreviations designating configurations of alkene moieties (replacing *cis* and *trans* respectively). The title structure may contain one or more types of heterocycles in combination. This group of compounds exhibits anticancer activity *in vitro* against the human tumor KB cell line and the murine cell line P388 and *in vivo* anticancer activity against mice injected with P388 leukemia.

The title CPI-oligopeptides exhibit extremely high potencies against human tumor KB cells (Tables 1-3). In certain cases the cytotoxic potencies (TD_{50} of ca. 10^{-15} M) exceed that of any other agent reported to date. They also have proven efficacy against i.p. implanted tumors in animals, affording increased life span of up to 57%. The title compounds in the latter tests showed no evidence of the delayed death syndrome that is the most serious limitation of drugs more directly related structurally to CC-1065. Thus CC-1065 proved to be too toxic for clinical usage, and analogs that may avoid the delayed death syndrome are currently undergoing clinical trials. FCE 24517 has similarly yet to obtain approval for clinical use.

Another distinct advantage of the title compounds over CC-1065, FCE 24517 and related structures is their ability to recognize and covalently bind to different DNA sequences (Tables 5, 6). CC-1065 and FCE 24517 are both strictly AT recognizing. Moreover FCE 24517 shows no evidence of DNA alkylation (Arcamone *et al.*, 1989). In contrast the title compounds alkylate DNA strongly, like CC-1065, but unlike the latter are capable via the variable oligopeptide of recognizing and binding covalently to quite different sequences. This is of significance therapeutically since it has been demonstrated that clinically effective alkylators like cyclophosphamide, nitrosoureas and mitozolomide show marked DNA sequence selectivity for alkylation of the central guanine of runs of three or more guanines (Hartley, J.A., Gibson, N.W., Kohn, K.W., Mattes, W.B., *Cancer Research*, 1986, 46, 1943). Effective targeting of such G-rich regions of the genome, particularly some oncogenes may provide a basis for the unusual potency of such sequence-directed alkylators (Hartley, J.A., Lown, J.W., Mattes, W.B., Kohn, K.W., *Acta Oncologica*, 1988, 27, 503). The combination of the CPI alkylating moiety with the versatile DNA-sequence reading capability of the oligopeptide side-chain confers exceptional cytotoxic

potency on these agents exceeding in the case of YW-059, for example, that of any other reported natural or synthetic agents.

Detailed Description of the Preferred Embodiments

5 Compounds according to the present invention demonstrate anticancer activity. The following data summarized in Tables 1-3 documents the *in vitro* evidence for extremely high cytotoxic potency of the new CPI-oligopeptides against KB human nasopharyngeal tumor cells.

10 In the case of YW-059 (Table 2) the cytotoxic potency exceeds that of any other agent, natural or synthetic, reported to date. Table 3 presents cytotoxicity data on representative imidazole-bearing CPI-oligopeptides, i.e. capable of recognizing and binding covalently to mixed DNA

15 sequences quite distinct from the strictly limited AATT recognition by either CC-1065 or FCE 24517. Table 4 presents animal data on the new drugs confirming significant anticancer activity is exhibited also *in vivo*.

Table 1. Cytotoxicity Data on New CPI-Oligopeptides

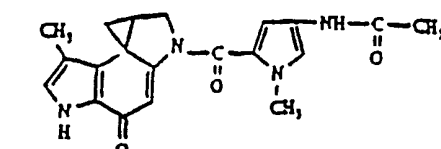
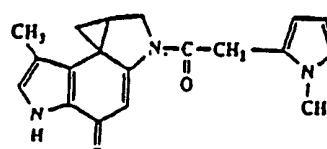
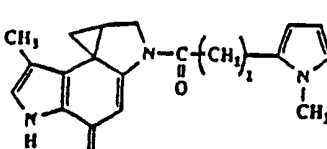
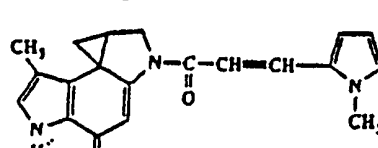
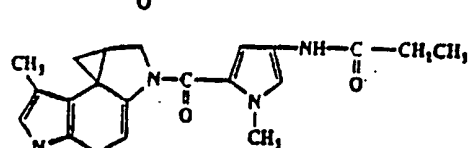
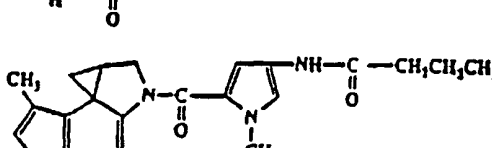
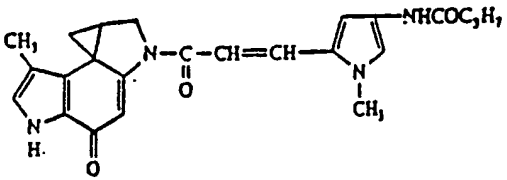
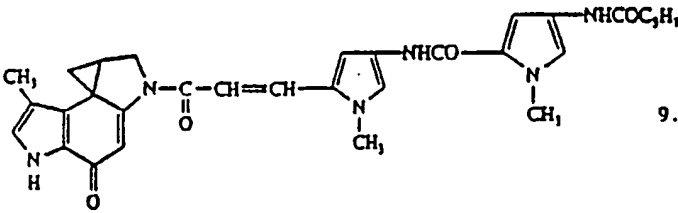
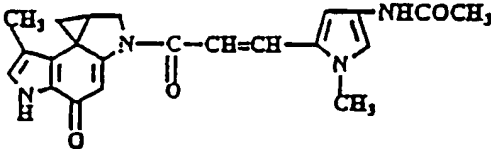
Code Name	Structure	KB cells, toxicity $TD_{50}/\mu g\ mL^{-1}$
YW-031		3.24×10^{-5}
YW-032		3.8×10^{-2}
YW-033		2.63×10^{-1}
YW-034		2.75×10^{-4}
YW-035		1×10^{-3}
YW-036		4.71×10^{-4}
Adriamycin	7	1.1×10^{-2}

Table 2. Cytotoxicity Data on New CPI-Oligopeptides

Code Name	Structure	KB cells, toxicity $TD_{50}/\mu g\ mL^{-1}$
YW-052		7.63×10^{-10}
YW-053		9.50×10^{-10}
YW-059		1.5×10^{-7}
CC-1065 - Adriamycin		1×10^{-4} 5×10^{-3}

*Equivalent to TD_{50} of ca. 10^{-15} M, i.e. the most potent cytotoxic agents ever reported.

Table 3. Cytotoxicity Data on New CPI-Oligopeptides

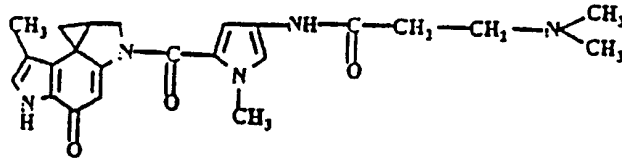
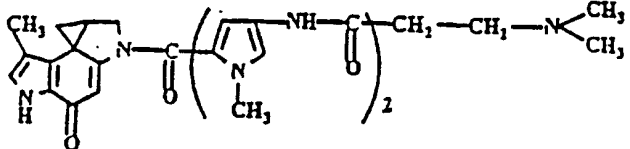
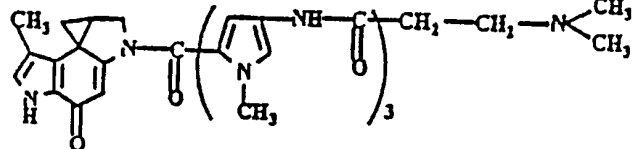
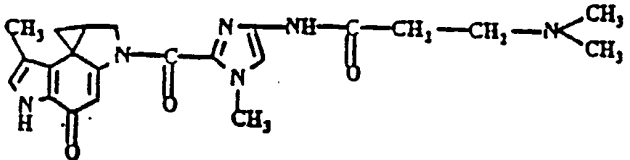
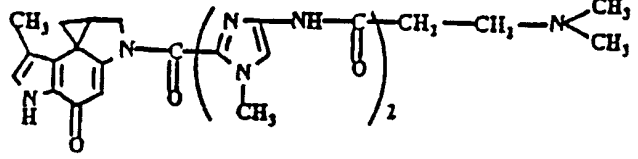
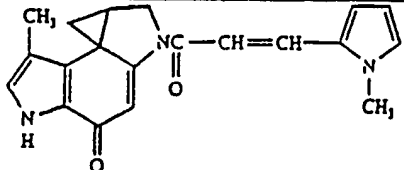
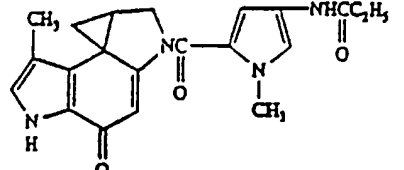
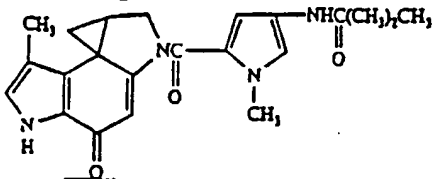
Code Name	Structure	KB cells, toxicity $TD_{50}/\mu g\ mL^{-1}$
YW-60		0.184
YW-61		2.49
YW-62		1.37
YW-63		0.385
YW-64		2.52

Table 4. In vivo Anticancer Activity of CPI-Oligopeptides

Compound	Chemical Structure	M.W.	<i>In vivo</i> ILS (%) P388 (ip)	Cytotoxicity IC ₅₀ (μg/mL) (KB cell)
YW-34		333.4	37.9 (1 mg/kg)	0.00027
YW-35		379.4	56.5 (0.1 mg/kg)	0.001
YW-36		393.5	24.6 (1/7 cure) (0.3 mg/kg)	0.00047

1) Number of 30 day survivors is not included to calculate I.L.S. value.

In contrast to FCE 24517, clear evidence of site and sequence selective DNA alkylation was obtained for the new CPI-oligopeptides by polyacrylamide gel electrophoresis (PAGE). Figure 1 displays the PAGE for YW-30, YW-32, YW-33, and YW-34 compared with CC-1065. Table 5 quantifies and compares the alkylation intensities at different sites for the CPI-oligopeptides with CC-1065. The PAGE results for some of the most potent drugs YW-052 and YW-053 are shown in Figure 2 and tabulated for the primary and secondary alkylation sites in Table 6. A detailed analysis of the frequency of occurrence of bases flanking the prominent alkylation sites for YW-052 and YW-053 compared with CC-1065 is given in Table 7. These data provide evidence of the main cellular event that gives rise to the expression of anticancer properties of the new drugs and how they differ in detail from CC-1065.

Table 5. Summary of Alkylation Sites and Alkylation Intensity of CC-1065 and its Analogs

Alkylation Intensity at:										
Compound	ATAA*	AATA*	ATAA*	GATA*	TTAA*	GTTA	TATA	TTTA	CTTA	GAAA
CC-1065	1.47	1.08	.96	.80	.80	1.20	1.02	1.09	.61	.35
YW-30	.76	1.27	.85	1.38	.55	.95	.83	.50	.44	.66
YW-32	.88	1.35	1.01	1.35	.71	1.03	1.05	.63	.61	.79
YW-33	.95	1.42	1.11	1.41	.84	1.27	1.40	.97	.68	.84
YW-34	.71	1.30	.84	1.09	.52	.73	.77	.49	.44	.55

*Denotes Alkylated adenines at 3' end

Table 6. Alkylation Sites of CC-1065 and its
Analogues

Compound	Alkylation Sites* (5'-3')	
	Strong	Moderate
CC-1065	TTT <u>A</u> , GAT <u>A</u> , ATA <u>A</u> , AAT <u>A</u> , TAT <u>A</u> , TTA <u>A</u> , GTT <u>A</u> , CTT <u>A</u> , ATA <u>A</u>	GGA <u>A</u>
YW-52	TTT <u>A</u> , GAT <u>A</u> , ATA <u>A</u> , AAT <u>A</u> , TAT <u>A</u> , TTA <u>A</u> , GTT <u>A</u> , CTT <u>A</u> , ATA <u>A</u>	ATG <u>G</u> , GGG <u>A</u>
YW-53	TTT <u>A</u> , TAT <u>A</u> , GTT <u>A</u> , AAT <u>A</u> , TTA <u>A</u> , AAT <u>G</u> , GAT <u>A</u> , ATA <u>A</u> , ATA <u>A</u>	CTT <u>A</u> , GAA <u>A</u>

*Alkylation sites are represented by underlined bases
at the 3' end.

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Table 7. Analysis of the DNA Sequence Flanking the Prominent Alkylation Sites of CC-1065 and its Analogs

		Frequency of Occurrence (%)							
Compound		-3	-2	-1	A*	G*	+1	+2	+3
CC-1065	A	30	40	40	100	0	30	30	20
	T	30	60	60			50	30	30
	G	10	-	-			20	40	40
	C	30	-	-			-	-	10
	A/T	60	100	100			80	60	50
YW-52	A	36	27	27	91	9	46	36	27
	T	27	64	55			36	37	27
	G	27	9	18			18	27	36
	C	10	-	-			-	-	10
	A/T	63	91	82			82	73	54
YW-53	A	36	45	36	91	9	27	27	27
	T	27	55	64			45	27	27
	G	27	-	-			28	37	37
	C	10	-	-			-	9	9
	A/T	63	100	100			82	54	54

*Represents site of alkylation. Columns to the left and right of alkylated site(s) represent bases to the 5' and 3' sides, respectively.

The compounds of the present invention are useful as anticancer agents. An effective amount of one or more of the present compounds is administered to a patient preferably in the presence of a pharmaceutically acceptable carrier or diluent. Pharmaceutically compatible binding agents and/or adjuvant materials can also be included.

The compounds according to the present invention can be administered by any route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, rectally or topically in a liquid or solid form. For injection purposes, the medium used may be a sterile liquid. As an injection medium, it is preferred to use water which contains conventional stabilizing agents, solubilizing agents and/or buffers. Desirable additives include but are not limited to tartrate and borate buffers, ethanol, dimethylsulfoxide, complex forming agents (for example ethylenediamine tetracetic acid), high molecular weight polymers (for example liquid polyethylene oxide) for viscosity regulation or polyethylene derivatives of sorbitan anhydrides. Solid carrier materials include but are not limited to starch, lactose, mannitol, methylcellulose, talc, highly dispersed silicic acid, high molecular weight fatty acids (for example stearic acid), gelatin, agar, calcium phosphate, magnesium stearate, animal and vegetable fats or solid high molecular weight polymers (for example polyethylene glycol).

The present compounds can be administered in dosages and amounts which are conventional in the art. The compounds can be used at a dosage range of about 1-200 mg/kg total body weight/day. The dosages may be administered at once or may be divided into a number of smaller doses to be administered at varying intervals of time.

Brief Description of the Drawings

Figure 1 shows the results of PAGE for YW-30, YW-32, YW-33, YW-34 and CC-1065.

Figure 2 shows the results of PAGE for CC-1065, YW-052 and YW-053.

Figure 3 shows synthetic routes for cyclopropylpyrroloindole-oligopeptide anticancer agents.

Preferred embodiments of the invention are exemplified in the following examples which are in no way to be construed as limiting the scope of the present invention.

Example 1

1, 2, 8, 8a-Tetrahydro-7-methyl-2-(4-butyramido-N-methyl-2-pyrroleacryloyl)-cyclopropa[c]-pyrrolo[3, 2, e]indole-4-(5 H)-one (CP7-19)

Compound CP7-19 of the formula X, where R = *trans*-CH=CH; Het=N-methylpyrrole; m = 1; n = 0; A = Butyramido, was prepared. 10% Pd/C (30 mg) and ammonium formate (30 mg) were added to 5-benzyloxy-3-*tert*-butyloxycarbonyl-1-chloromethyl-8-methyl-1,2-dihydro-3Hpyrrolo[3,2-e]indole (30 mg), which was synthesized using the reported methods (Ref. D. L. Boger and R. S. Coleman, *J. Am. Chem. Soc.* 1988, 110, 4796-4807), dissolved in a solution of tetrahydrofuran and methanol (0.8 ml, 1/1, v/v) and the reaction mixture was stirred at room temperature for 20 min. The solid was removed by filtration and water (2 ml) was added. The product was extracted with ethyl acetate (10 ml x 3) and the organic phase was dried over sodium sulfate. The solvent was removed *in vacuo* to generate an oil CP7-13. Without further purification, the oil CP7-13 was treated with anhydrous 3 N hydrochloric acid in ethyl acetate (3 ml) at room temperature for 40 min. The solvent was removed *in vacuo* to give an unstable intermediate, CP7-14. Without further purification, CP7-14 was dissolved in dimethylformamide (1 ml) under

nitrogen. 4-Butyramido-N-methyl-2-pyrroleacrylic acid (CP7-6) (16.6 mg) and 1-[(3-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (67.6 mg) were added sequentially. The reaction mixture was stirred in dark at room temperature for 2 days. The mixture was purified on silica plate eluting with a mixture of acetone and ethylacetate (1:1, v/v) to give an unstable CP7-17. Without further purification, CP7-17 was dissolved in a mixture of acetonitrile, triethylamine and water (0.6 ml of each) and the reaction mixture was stirred for 30 min under nitrogen. The solvent was removed by a high vacuum pump and the residue was dissolved in acetone. The mixture was then purified on a silica plate eluting with a mixture of acetone and ethyl acetate (1:1, v/v). 9.4 mg (32 % yield) of a yellow powder was obtained. ¹H NMR (acetone-d₆, ppm): 10.48 (br s, 1H, NH), 8.97 (br s, 1H, NH), 7.63-7.58 (d, 1H, J=15.5 Hz, CHCH), 7.34 (d, 1H, J=2.0 Hz, C5'-H), 6.88 (br m, 1H, C6-H), 6.72 (br s, 1H, C3-H), 6.67 (d, 1H, J=2.0 Hz, C3'-H), 6.58-6.62 (d, 1H, J=15.5 Hz, CHCH), 4.31-4.27 (d, 1H, J=10.5 Hz, NCHH), 4.21-4.16 (dd, 1H, J=4.5, 10.5 Hz, NCHH), 3.76 (s, 3H, NCH₃), 3.08-3.03 (m, C8a-H), 2.27-2.21 (t, 2H, J=7.5 Hz, CH₂CH₂CH₂), 1.97-1.93 (dd, 1H, J=4.5, 7.5 Hz, C8-HH), 1.7-1.59 (m, 2H, CH₂CH₂CH₂), 1.28-1.25 (t, 1H, J=4.5 Hz, C8-HH), 0.94-0.89 (t, 3H, J=7.5 Hz, CH₃CH₂CH₂). FABHRMS calcd for C₂₄H₂₆N₄O₃H 419.2083, found 419.2092 (100%).

Example 2

1, 2, 8, 8a-Tetrahydro-7-methyl-2-[4-(4-butyramido-N-methyl-2-pyrrolecarboxyamido)-N-methyl-2-pyrroleacryloyl]cyclopropa[c]-pyrrolo[3, 2, e]indole-4-(5H)-one (CP7-20)

Compound CP7-20 of the formula X, where R *trans*-CH=CH; Het=N-methylpyrrole; m = 2; n = 0; A = butyramido, was prepared from 5-benzyloxy-3-*tert*-butyloxycarbonyl-1-chloromethyl-8-methyl-1,2-dihydro-3H-pyrrolo[3,2-e]indole (30 mg) and 4-(4-butyramido-N-methyl-2-

pyrrolecarboxyamido)-N-methyl-2-pyrroleacrylic acid (25 mg) using the same method as described for CP7-19. The product is a yellow powder (15% yield). ¹H NMR (acetone-d₆, ppm): 10.54 (br s, 1H, NH), 9.25 (br s, 1H, NH), 8.97 (br s, 1H, NH), 7.64-7.60 (d, 1H, J=15.5 Hz, CHCH), 7.42 (d, 1H, J=2.0 Hz, C5'-H), 7.17 (d, 1H, J=2.0 Hz, C5'-H), 6.89 (br m, 1H, C6-H), 6.83 (d, 1H, J=2.0 Hz, C3'-H), 6.80 (d, 1H, J=2.0 Hz, C3"-H), 6.73 (br s, C3-H), 6.58-6.63 (d, 1H, J=15.5 Hz, CHCH), 4.32-4.28, (d, 1H, J=10.5 Hz, NCHH), 4.22-4.17 (dd, 1H, J=4.5, 10.5 Hz, NCHH), 3.90 (s, 3H, NCH₃), 3.78 (s, 3H, NCH₃), 3.09-3.03 (m, C8a-H), 2.25-2.20 (t, 2H, J=7.5 Hz, CH₂CH₂CH₂), 1.97-1.94 (dd, 1H, J=4.5, 7.5 Hz, C8-HH), 1.70-1.58 (m, 2H, CH₂CH₂CH₂), 1.29-1.27 (t, 1H, J=4.5 Hz, C8-HH), 0.94-0.89 (t, 3H, J=7.5 Hz, CH₃CH₂CH₂). FABHRMS calcd for C₃₀H₃₂N₆O₄H 541.2563, found 541.2563 (100%).

Example 3

1, 2, 8, 8a-Tetrahydro-7-methyl-2-(N-methyl-2-pyrroleacetoxycyclopropa-[c]-pyrrolo[3, 2, e]indole-4-(5 H)-one (CP5-13).

Compound CP5-13 of the formula X, where R = CH₃; Het=N-methylpyrrole; m = 1; n = 0; A = H, was prepared. 5-Benzyloxy-3-tert-butyloxylvcarbonyl-1-chloromethyl-8-methyl-1,2-dihydro-3H-pyrrolo[3,2-e]indole (10 mg, 23 umol) was dissolved in 3 N HCl in ethyl acetate (2 ml) and the solution was stirred at ambient temperature for 40 min. The solvent was removed and dichloromethane (2 ml) was added. Dichloroniethane was removed and the residue was dissolved in dimethylformamide (0.5 ml). N-methyl-2-pyrroleacetic acid (5 mg, 36 umol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (40 mg, 208 umol) and sodium hydrogen carbonate (10 mg) were added sequentially. The reaction mixture was stirred at ambient temperature overnight and then purified by flush column chromatography eluding with a mixture of ethyl acetate and hexane (1:3 to 1:1, v/v). White solid CP5-9

(10.2 mg, 22.8 μmol , 97%): mp 188-190 $^{\circ}\text{C}$; ^1H NMR (CDCl_3): 8.18 (br s, 1H, NE), 8.04 (s, 1H, C4-H), 7.47-7.34 (m, 5H, C_6H_5), 6.96 (s, 1H, C7-H), 6.63 (s, 1H, C5'-H), 6.10 (s, 2H, C3'-H, C4'-H), 5.22-5.13 (q, 2H, $J=3.0$, 12 Hz, $\text{C}_6\text{H}_5\text{CH}_2\text{O}$), 4.45-4.41 (d, 1H, $J=11.0$ Hz, NCHH), 4.0-3.93 (m, 1H, $\text{CH}_2\text{ClCHCH}_2$), 3.85-3.80 (m, 3H, COCH_2 , CHHC1), 3.70 (s, 3H, NCH_3), 3.40-3.33 (t, 1H, $J=10.5$ Hz, CHHC1), 2.40 (s, 3H, ArCH_3); EIHRMS calcd for $\text{C}_{26}\text{H}_{26}\text{ClN}_3\text{O}_2$, 447.1716 found 447.1708, was obtained.

To a solution of CP5-9 (5.1 mg, 11.4 μmol) in methanol and THF (0.6 ml, 1:1, v/v) was added ammonium formate (10 mg) and 10% Pd/C (10 mg) and the reaction mixture was stirred at ambient temperature for 20 min. The mixture was filtered and the filtrate was extracted with ethyl acetate (10 ml x 2). The solvent was removed in vacuo. To the residue were added acetonitrile (1 ml), triethylamine (0.2 ml) and water (0.2 ml) and the solution was stirred at ambient temperature for 30 min. The solvent was removed and the salt in flask was washed away using water (2 ml x 3). The flask was dried and ether was added. The solid product was filtered and washed with more ether. 1.5 mg (4.67 μmol , 41% yield) of a gray powder was obtained: ^1H NMR (acetone- d_6): 6.87 (s, 1H, C6-H), 6.62-6.60 (t, 1H, $J=2.0$ Hz, C5'-H), 5.91-5.89 (t, 1H, $J=3.0$ Hz, C4'-H), 5.88-5.86 (m, 1H, C3'-H), 4.32-4.29 (d, 1H, $J=11.0$ Hz, NCHH), 4.21-4.16 (m, 1H, NCHH), 3.88-3.86 (d, 2H, $J=5.0$ Hz, COCH_2), 3.56 (s, 3H, NCH_3), 3.08-3.04 (m, 1H, C8a-H), 2.01 (d, 3H, $J=1.0$ Hz, ArCH_3), 1.92-1.88 (dd, 1H, $J=4.0$, 7.0 Hz, C8-HH), 1.23-1.20 (t, 1H, $J=4.5$ Hz, C8-HH); EIHRMS calcd for $\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_2$, 321.1479 found 321.1462.

Example 4

1, 2, 8, 8a-Tetrahydro-7-methyl-2-(N-methyl-2-pyrrolepropionyloxy)cyclopropa(c)-pyrrolo[3, 2, e]indole-4-(5 H)-one (CP5-14).

Compound CP5-14 of the formula X, where $R = CH_2CH_2$; Het=N-methylpyrrole; $m = 1$; $n = 0$; $A = H$, was prepared. Using the same method as described for CP5-9, from 5-benzyloxy-3-tert-butyloxycarbonyl-1-chloromethyl-8-methyl-1,2-dihydro-3H-pyrrolo[3,2-e]indole and N-methyl-2-pyrrolepropionic acid, 5-benzyloxy-1,2-dihydro-1-chloroxymethyl-8-methyl-3(N-methyl-2-pyrrolepropionyloxy)-3H-pyrrolo[3,2-e]indole (CP5-10) was synthesized in the yield of 60% as a white solid: mp 159-161°C; 1H NMR (acetone-d₆): 8.09 (s, 1H, C4-H), 7.58-7.30 (m, 5H, C₆H₅), 7.08 (s, 1H, C7-H), 6.56-6.55 (t, 1H, J=2.0 Hz, C5'-H), 5.90-5.88 (dd, 1H, J=0.5, 2.5 Hz, C4'-H), 5.88-5.86 (m, 1H, C3'-H), 5.23 (s, 2H, C₆H₅CH₂O), 4.30-4.18 (m, 2H, NCH₂), 4.33-3.96 (m, 1H, CH₂ClCHC₂), 3.89-3.84 (dd, 1H, J=2.5, 10.5 Hz, CHHC1), 3.70 (s, 3H, NCH₃), 3.53-3.46 (t, 1H, J=10., 5 Hz, CHHC1), 2.96-2.79 (m, 4H, CH₂CH₂), 2.40 (s, 3H, ArCH₃); EIHRMS calcd for C₂₇H₂₈ClN₃O₂, 461.1873 found 461.1873.

By the treatment of CP5-10 in a similar procedure as described for CP5-13 except that sodium hydride in N,N-dimethylformamide (0.2 ml) and tetrahydrofuran (0.8 ml) was used for the final cyclization reaction, 46% yield of a grey solid CP5-14 was obtained: 1H NMR (DMF-d₆): 6.95 (s, 1H, C6-H), 6.66-6.64 (t, 1H, J=2.0 Hz, C5'-H), 5.90-5.88 (t, 1H, J=3.0 Hz, C4'-H), 5.85-5.82 (m, 1H, C3'-H), 4.26-4.22 (d, 1H, J=11.0 Hz, NCH-H), 4.20-4.17 (m, 1H, NCHH), 3.60 (s, 3H, NCH₃), 3.15-2.90 (m, 1H, C8a-H), 2.30 (d, 3H, J=1.0 Hz, ArCH₃), 1.94-1.90 (dd, 1H, J=4.0, 7.0 Hz, C8-HH), 1.25-1.22 (t, 1H, J=4.5 Hz, C8-HH); EIHRMS calcd for C₂₀H₂₁N₃O₂, 335.1636 found 335.1618.

Example 5

1, 2, 8, 8a-Tetrahydro-7-methyl-2-(4-acetamido-N-methyl-2-pyrrolocarboxy)cyclopropa[cl-pyrrolo(3, 2, e)indole-4-(5 H)-one (CP4-19).

Compound CP4-19 of the formula X, where $R = O$; Het=N-methylpyrrole; $m = 1$; $n = 0$; $A =$ acetamido, was prepared.

5-Benzyloxy-3-tert-butyloxycarbonyl-1-chloromethyl-8-methyl,2-dihydro-3H-pyrrolo[3,2-e]indole (20 mg, 47 μ mol) was dissolved in 3 N HCl in ethyl acetate and the solution was stirred at ambient temperature for 40 min. The solvent was removed and dichloromethane (2 ml) was added. Dichloromethane was removed and the residue was dissolved in dimethylformamide (1 ml). 4-Acetamido-N-methyl-2-pyrrolicarboxylic acid (CP4-5) (10.2 mg, 56 μ mol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (45 mg, 234 μ mol) and sodium hydrogen carbonate (20 mg) were added sequentially. The reaction mixture was stirred at ambient temperature overnight and then purified by flush column chromatography using ethyl acetate as an eluent. A grey powder (18.4 mg, 80% yield) was obtained as product CP4-11: mp 143-145 $^{\circ}$ C; 1 H NMR (500 MHz, CDCl_3): 8.15 (br s, 1H, NH), 8.00 (br s, 1H, NH), 7.60 (br s, 1H, C4-H), 7.43-7.32 (m, 5H, C_6H_5), 7.24 (s, 1H, C5'-H), 6.97 (s, 1H, C7-H), 6.42 (s, 1H, C3'-H), 5.22-5.11 (q, 2 H, $\text{J}=6.6$, 25.8 Hz, PhCH_2O), 4.52-4.50 (d, 1 H, NCHH , $\text{J}=6.6$ Hz), 4.31-4.27 (dd, 1 H, $\text{J}=4.8$, 6.6 Hz, NCHH), 3.83-3.78 (m, 5 H, CHHCl , $\text{CH}_2\text{ClCHCH}_2$, NCH_3), 3.42-3.38 (t, 1H, $\text{J}=6.6$ Hz, CHHCl), 2.40 (s, 3 H, ArCH_3), 2.17 (s, 3H, CH_3CO); EIHRMS calcd for $\text{C}_{27}\text{H}_{27}\text{N}_4\text{O}_3\text{Cl}$, 490.1774, found 490.1765.

To a solution of CP4-11 (20.6 mg, 42 μ mol) in methanol and tetrahydrofuran (0.6 ml, 1:1, v/v) was added ammonium formate (20 mg) and 10% Pd/C (20 mg) and the reaction mixture was stirred at ambient temperature for 20 min. The mixture was filtered and water (3 ml) was added to the filtrate. The mixture was extracted with ethyl acetate (10 ml x 2). The solvent was removed in vacuo. To the residue were added acetonitrile (0.4 ml), triethylamine (0.2 ml) and water (0.2 ml) and the solution was stirred at ambient temperature for 20 min. The solvent was removed and the salt in flask was washed away using water (2 ml x 3). Ethyl ether was added and the solid product was filtered and washed with more ether. A gray powder product (10.5 mg, 69% yield) was obtained: 1 H NMR (acetone- d_6): 10.10 (br s, 1H, NH), 8.74 (br s, 1H,

NH), 7.28 (s, 1H, C5'-H), 6.82 (s, 1H, C6-H), 6.45 (s, 1H, C3'-H), 6.17 (s, 1H, C3-H), 4.22-4.17 (dd, 1H, NCHH, J=4.5, 11 Hz), 4.08-4.05 (d, 1H, J=11 Hz, NCHH), 3.77 (s, 3 H, NCH₃), 2.93-2.87 (m, 1H, C8a-H), 2.02-1.97 (m, 7H, ArCH₃, CH₃CO, C8-HH), 1.40-1.33 (q, 1H, J=5.0, 10 Hz, C8-HH), EIHRMS calcd for C₂₀H₂₀N₄O₃, 364.1537, found 364.1580.

Example 6

1, 2, 8, 8a-Tetrahydro-7-methyl-2-(4-(4-[4-(3-dimethylaminopropionamido)-N-methylpyrrole-2-carboxyamido]-N-methylpyrrole-2-carboxyamido)-N-methylpyrrole-2-carboxylcyclopropa(c)-pyrrolo[3, 2, e]indole-4-(5 H)-one (CP338)

Compound CP3-38 of the formula X, where R = 0; Het=N-methylpyrrole; m = 3; n = 0; A = 3-dimethylaminopropionamido, was prepared. Pd/C (25 mg) and ammonium formate (25 mg, 397 umol) were added to 5-benzyloxy-3-tert-butyloxycarbonyl-1-chloromethyl-8-methyl-1, 2-dihydro-3H-pyrrolo[3,2-e]indole (CP3-28, 20 mg, 47 umol) dissolved in a solution of tetrahydrofuran and methanol (0.6 ml, 1/1, v/v) and the reaction mixture was stirred at room temperature for 20 min. The solid was removed by filtration and water (2 ml) was added to the filtrate. The mixture was extracted with ethyl acetate (5 ml x 3) and the organic phase was dried over sodium sulfate. The solvent was removed in vacuo and a colorless solid, CP3-29, was obtained. Without further purification, CP3-29 was treated with anhydrous 3 N hydrochloric acid in ethyl acetate at room temperature for 40 min. The solvent was removed in vacuo to give an unstable CP3-30. Dimethylformamide (0.8 ml), sodium hydrogen carbonate (21 mg, 250 umol), 4-(4-[4-(3-dimethylaminopropionamido)-N-methylpyrrole-2-carboxyamido]-N-methylpyrrole-2-carboxyamido)-N-methylpyrrole-2-carboxylic acid (CP3-20) (21 mg, 50 umol) and I-[(3dimethylamino)propyl]3-ethylcarbodiimide hydrochloride (48 mg, 250 umol) were added sequentially to

CP3-30 under nitrogen. The reaction mixture was stirred in dark at room temperature overnight. The product CP3-33 was purified on silica gel plate using methanol and acetone (1/3, v/v) as an eluent and washed off the silica using dimethylformamide and tetrahydrofuran (1/4, v/v). 8 mg (11 umol, 23% yield) of unstable 31 was obtained. To a solution of CP3-33 (8 mg, 11 umol) in dimethylformamide and tetrahydrofuran (0.8 ml, 1/3, v/v) was added sodium hydride (4 mg, 100 umol, 60% dispersion in mineral oil) at 0 °C under nitrogen. The reaction mixture was stirred at 0 °C for 1 h. The product was purified on silica gel plate using methanol and acetone (1/3, v/v) as an eluent and washed off the silica gel using dimethylformamide and tetrahydrofuran (1/4, v/v). The product CP3-38 (2.7 mg, 4 umol, 32% yield) was obtained: ¹H NMR (DMF-d₇): 11.50 (s, 1H, NH), 10.10 (s, 1H, NH), 10.02 (s, 1H, NH), 9.97 (s, 1H, NH), 7.55 (d, 1H, J = 1.8 Hz, py-CH), 7.31 (d, 1H, J=1.8 Hz, py-CH), 7.23 (d, 1H, J=1.8 Hz, py-CH), 7.20 (d, 1H, J=1.8 Hz, py-CH), 6.97-6.95 (m, 2 H, C6-H, py-CH), 6.82 (d, 1H, J=1.8 Hz, py-CH), 6.23 (s, 1H, C3-H), 4.33-4.28 (dd, 1H, J=4.5, 11.0 Hz, C1-HH), 4.18-4.15 (d, 1H, J=11.0 Hz, C1-HH), 3.95 (s, 3 H, NCH₃), 3.92 (s, 3 H, NCH₃), 3.83 (s, 3 H, NCH₃), 3.10 (m, 1H, C8a-H), 2.57-2.54 (t, 2 H, J=6.0 Hz, COCH₂), 2.43-2.39 (t, 2 H, J=6.0 Hz, CH₂N), 2.18 [s, 6 H, 2XNCH₃, 2:05 (s, 3 H, ArCH₃), 2.0 (m, partially obscured by ArCH₃, 1H, C8-HH), 1.43-1.41 (t, 1H, J=4.5 Hz, C8-HH); FABHRMS calcd for C₃₅H₃₉N₉O₅ (M + H) 666.3152, found 666.3156.

Example 7

1, 2, 8, 8a-Tetrahydro-7-methyl-2-(4-[4-(3-dimethylaminopropionamido)-N-methylimidazole-2-carboxyamido]-N-methylimidazole-2-carboxy)-cyclopropa[c]pyrrolo[3, 2, e]indole-4-(5 H)-one (CP3-40).

Compound CP3-40 of the formula X, where R = 0; Het=N-methylimidazole; m = 2; n 0; A = 3-dimethylaminopropionamido, was prepared from 4-[4-(3-dimethylaminopropionamido)-N-methylimidazole-2 -

carboxamido]-N-methylimidazole-2-carboxylic acid and 5-benzyloxy-3-~~tert~~-butyloxycarbonyl-1-chloromethyl-8-methyl-1,2-dihydro-3H-pyrrolo[3,2-e]indole CP3-28 using the same procedure as that described for CP3-38 (13% yield from CP3-28). ¹H NMR (DMF-d₇): 11.55 (s, 1H, NH), 10.65 (s, 1H, NH), 9.64 (s, 1H, NH), 7.69 (s, 1H, im-CH), 7.56 (s, 1H, im-CH), 7.00 (d, 1H, J=2.0 Hz, C6-H), 6.97 (s, 1H, C3-H), 4.84-4.80 (d, 1H, J=11.0 Hz, C1-HH), 4.54-4.48 (dd, 1H, J=11.0 Hz, C1-HH), 4.09 (s, 3 H, NCH₃), 4.00 (s, 3 H, NCH₃), 3.20 (m, 1 H, C8a-H), 2.61-2.56 (m, 4 H, CH₂CH₂), 2.20 [s, 6 H, 2XNCH₃], 2.05 (s, 3 H, ArCH₃), 2.02-1.98 (dd, 1 H, C8-HH, J=4.5, 7.5 Hz), 1.41-1.38 (t, 1H, C8-HH, J=4.5 Hz); FABHRMS calcd for C₂₇H₃₂N₆O₄ (M + H) 546.2577, found 546.2583.

15 **Example 8**

1, 2, 8, 8a-Tetrahydro-7-methyl-2-[4-(4-butyramido-N-methoxymethylpyrrole-2-carboxyamido)-N-methoxymethylpyrrole-2-carboxylcyclopropa(c)]-pyrrolo[3, 2-e] lindole-4-(5 ff)-one (CP8-26)

20 Compound CP8-26 of the formula X, where R = 0; Het = N-methoxymethylpyrrole; m=2; n = 0; A = butyramido, was prepared. 5-Benzyloxy-3-~~tert~~-butyloxycarbonyl-1-chloromethyl-8-methyl-1,2-dihydro-3H-pyrrolo[3,2-e]indole CP8-14 (33 mg, 77.5 umol) was dissolved in 3 N HCl in
25 ethyl acetate (3 ml) and the solution was stirred at ambient temperature for 40 min. The solvent was removed and dichloromethane (2 ml) was added. Dichloromethane was removed and the residue was dissolved in dimethylformamide (1 ml), 4-(4-butyramido-N-methoxymethylpyrrole-2-
30 carboxamido)-N-methoxymethylpyrrole-2-carboxylic acid CP8-11 (31 mg, 79.2 umol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (74 mg) and sodium hydrogen carbonate (15 mg) were added sequentially. The reaction mixture was stirred at ambient temperature
35 overnight and then purified by flash column chromatography using ethyl acetate and hexane (2: 1, v/v) as an eluent to

afford 5-benzyloxy-1-chloromethyl-8-methyl-3-[4-(4-butyramido-N-methoxymethylpyrrole-2-carboxyamido)-N-methoxymethylpyrrole-2-carboxy]-1, 2-dihydro-3H-pyrrolo[3, 2-e]indole CP8-18 (38 mg, 54 μ mol, 70% yield): ^1H NMR (CDCl₃): 8.57 (br s, 1H, NH), 8.35 (br s, 1H, NH), 7.64-7.33 (m, 8H, C4-H, 2xPy-H, C₆H₅), 6.94 (s, 1H, C7-H), 6.70 (s, 1H, Py-H), 6.57 (s, 1H, Py-H), 5.71-5.67 (d, 1H, J=9.0 Hz, CHHOCH₃), 5.53 (s, 2H, CH₂OCH₃), 5.22-5.05 (m, 3 H, CHHOCH₃, PhCH₂O), 4.48-4.44 (d, 1H, NCHH, J=11.0 Hz), 4.27-4.21 (t, 1H, J=9.5 Hz, NCHH), 3.79-3.71 (m, 2 H, CHHCl, CH₂ClCHCH₂), 3.39-3.33 (m, 4H, CHHCl, OCH₃), 3.25 (s, 3H, OCH₃), 2.38 (s, 3 H, ArCH₃), 2.282-2.24 (t, 2H, J=7Hz, CH₂CH₂CH₂), 1.73-1.64 (m, 2H, CH₂CH₂CH₂), 0.97-0.92 (t, 3H, J=7 Hz, CH₂CH₂CH₂). FABHRMS calcd for C₃₇H₄₀N₆O₆ClH 700.2776, found 700.2723.

To a solution of CP8-18 (38 mg, 54 μ mol) in methanol and THF (0.6 ml, 1:1, v/v) was added ammonium formate (20 mg) and 10% Pd/C (20 mg) and the reaction mixture was stirred at ambient temperature for 20 min. The mixture was filtered and water (3 ml) was added to the filtrate. The mixture was extracted with ethyl acetate (10 ml x 2). The solvent was removed in vacuo. To the residue were added acetonitrile (1 ml), triethylamine (1 ml) and water (1 ml) and the solution was stirred at ambient temperature for 1 h. The solvent was removed and the salt in the flask was washed away using water (2 ml X 3). Ethyl ether was added and the solid product was collected and washed with more ether to afford a grey powder CP8-26 (16.8 mg, 54% yield): ^1H NMR (acetone-d₇): 10.52 (br s, 1H, NH), 9.42 (br s, 1H, NH), 9.10 (br s, 1H, NH), 7.68-7.67 (d, 1H, J=1.5 Hz, Py-H), 7.40-7.39 (d, 1H, J=1.5 Hz, Py-H), 6.91-6.90 (m, 2H, C6-H, Py-H), 6.73-6.72 (d, 1H, J=1.5 Hz, Py-H), 6.14 (s, 1H, C3-H), 5.77-5.67 (m, 3H, CH₂OCH₃, CHHOCH₃), 5.27-5.23 (d, 1H, J=10.0 Hz, CHHOCH₃), 4.25-4.20 (dd, 1H, J=5.0, 11.0 Hz, NCHH), 4.12-4.08 (d, 1H, J=11.0 Hz, NCHH), 3.25 (s, 3H, OCH₃), 3.23 (s, 3H, OCH₃), 3.06-3.00 (m, 1H, CH₂CHCH₂), 2.25-2.20 (t, 2H, J=7 Hz, CH₂CH₂CH₂), 1.70-1.58 (m, 2H, CH₂CH₂CH₂), 1.40-1.37 (t, 1H,

J=4.5 Hz, C8-HH), 0.93-0.88 (t, 3H, J=7 Hz, CH₃CH₂CH₂), FABHRMS calcd for C₃₀H₃₄N₆O₆H 575.2618, found 575.2646.

Example 9

- 5 1, 2, 8, 8a-Tetrahydro-7-methyl-2-[4-(4-butyramido-N-methoxymethylimidazole-2-carboxyamido)-N-methoxymethylimidazole-2-carboxy]cyclopropa(c)-pyrrolo[3, 2-e]indole-4-(5 H)-one (CP8-27).

Compound CP8-27 of the formula X, where R = 0; Het=N-methoxymethylimidazole; m = 2; n = 0; A = butyramido, was
10 prepared. 5-Benzyloxy-1-chloromethyl-8-methyl-3-[4-(4-butyramido-N-methoxymethylimidazole-2-carboxyamido)-N-methoxymethylimidazole-2-carboxy]-1, 2-dihydro-3H-pyrrolo[3, 2-e]indole (CP8-19) was synthesized using the same method
15 as described for CP8-18 except that 4-(4-butyramido-N-methoxymethylimidazole-2-carboxyamido)-N-methoxymethylimidazole-2-carboxylic acid CP8-13 was used in the yield of 69%: ¹H NMR (CDCl₃): 10.65 (br s, 1H, NH), 9.03 (br s, 1H, NH), 8.28 (br s, 1H, NH), 7.82 (s, 1H, Im-H), 7.81 (s, 1H, Im-H), 7.66 (s, 1H, C4-H), 7.47-7.36 (m, 5H, C₆H₅), 6.99 (s, 1H, C7-H), 5.84-5.68 (m, 4H, 2XCH₂OCH₃), 5.20-5.05 (dd, 2H, J=11.0, 33.0 Hz, PhCH₂O), 4.89-4.85 (d, 1H, NCHH, J=11.5 Hz), 4.80-4.74 (dd, 1H, J=7.5, 11.0 Hz, NCHH), 4.02-3.80 (m, 2H, CHHCl, CH₂ClCH₂CH₂), 3.47-3.37 (m, 4H, CHHCl, OCH₃), 3.30 (s, 3H, OCH₃), 2.50-2.40 (t, 2H, J=7Hz, CH₃CH₂CH₂), 2.40 (s, 3H, ArCH₃), 1.87-1.74 (m, 2H, CH₃CH₂CH₂), 1.05-1.0 (t, 3H, J=7 Hz, CH₃CH₂CH₂), FABHRMS calcd for C₃₅H₃₉N₆O₆ClH 703.2759, found 703.2718.

30 CP8-27 was synthesized from CP8-19 (30 mg, 42 umol) using a similar method as described for CP8-26 as a grey powder (17.8 mg, 72% yield): ¹H NMR (acetone-d₆): 10.58 (br s, 1H, NH), 9.51 (br s, 1H, NH), 9.48 (br s, 1H, NH), 7.72 (s, 1H, Im-H), 7.65 (s, 1H, Im-H), 6.93-6.92 (m, 1H, C6-H), 6.81 (br s, 1H, C3-H), 5.88 (s, 3H, CH₂OCH₃), 5.77-5.62 (dd, 2H, J=11.0, 32.0 Hz, CH₂OCH₃), 4.69-4.65 (d, 1H,

35

J=11.0 Hz, NCHH), 4.51-4.46 (dd, 1H, J=5.0, 11.0 Hz, NCHH), 3.36 (s, 3H, OCH₃), 3.33 (s, 3H, OCH₃), 3.12-3.07 (m, 1H, CH₂CHCH₂), 2.43-2.38 (t, 2H, J=7Hz, CH₃CH₂CH₂), 2.20-2.17 (dd, 1H, J=4.5, 7.5 Hz, C8-HH), 1.76-1.63 (m, 2H, CH₃CH₂CH₂), 1.33-1.29 (t, 1H, J=4.5 Hz, C8-HH), 0.98-0.93 (t, 3H, J=7 Hz, CH₃CH₂CH₂), FABHRMS calcd for C₃₀H₃₄N₆O₆H 577.2523, found 577.2523.

Example 10. Cytotoxicity

KB human nasopharyngeal carcinoma cells, were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. KB cells were cultured in plastic tissue culture flasks and kept in an incubator at 37°C in humidified air containing 5% CO₂. These cells were free of Mycoplasma as determined with Hoechst 33258 stain.

The antiproliferative effect of compounds was determined using cell staining method by crystal violet.

One hundred-μl cultures were established at 10³ cells/well in 96-well tissue culture plates (Nunc, Roskilde, Denmark). After 24 h. 100 μl of the medium containing 2 times concentrated the compounds were added to the cell culture. After an additional 3 days culture, cells were fixed to 20 μl of 25% glutaraldehyde for 15 minutes and washed with water, dried and stained with 0.05% crystal violet in 20% methanol for 15 minutes. After washing with water and drying, crystal violet was extracted with 100μl of 50 mM NaH₂PO₄/ethanol (1:1 v/v) and OD₅₄₀ was measured by the multiscanphotometer (Corona, Tokyo, Japan). A linear relationship between cell number and the amount of dye binding was found over the range of cell densities observed all experiments. The concentration of compound that resulted in 50% of the absorbance of untreated cultures was determined by linear regression of the data.

Example 11. Antitumor Efficacy

All compounds were dissolved in dimethyl sulfoxide (DMSO), diluted in sterile 0.9% NaCl solution to 5% final

DMSO concentration. BALB/c X DBA/2F1 (CDF₁) mice were purchased from Japan CLEA. P388 lymphocytic leukemia cells were inoculated intraperitoneally (10⁶ cells) 1 day prior to treatment into 6 week-old male CDF₁ mice, and compounds were injected intraperitoneally in a total volume of 0.1 ml/10g mouse body weight. Efficacy for these models was expressed as the Increase in life span (ILS) of treated mice in comparison to untreated tumor-bearing mice. Maximum ILS obtained was shown in Table 4.

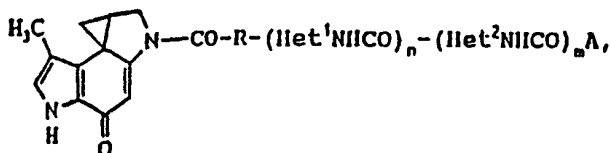
5 Experiment was completed on day 30 after tumor inoculation, and survivor on that day was evaluated as cured animal. Time to death (mean±SD.n=9) for untreated animals was 11.5±1.2 day.

10

CLAIMS

1. Compounds of the formula:

5



wherein,

Het¹ and Het² are individually selected from the group consisting of pyrrole, imidazole, triazole, thiophene, furan, thiazole, oxazole and pyrazole,

10

R is selected from the group consisting of a valence bond; a C₁-C₆ alkyl; a C₂-C₆ alkenyl; a C₂-C₆ alkynyl; and an ortho, meta or para linked aromatic group,

A is selected from the group consisting of a C₁-C₆ alkyl group; an amidine or derivative thereof; a guanidine; a secondary, tertiary or quaternary ammonium salt; and a sulfonium salt,

15

n is 0 to 3, and

m is 0 to 3.

2. The compounds according to claim 1, wherein R is selected from the group consisting of $-(CH_2)_n-$, wherein n = 1 to 6; $-CH=CH-$, E or Z; and $-C\equiv C-$.

20

3. The compound according to claim 1, wherein said aromatic group is selected from the group consisting of phenyl and naphthyl.

25

4. The compounds according to claim 1, wherein R is a divalent cycloalkane of formula C_pH_{2p-2} wherein p is 3 to 7.

7.

5. The compounds according to claim 1, wherein Het is N-alkylpyrrole, wherein the alkyl contains 1 to 4 carbon atoms.

6. The compounds according to claim 1, wherein Het is N-alkoxymethylpyrrole, wherein the alkoxy contains 1 to 4 carbon atoms.

7. The compounds according to claim 1, wherein Het is N-alkylimidazole, wherein the alkyl contains 1 to 4 carbon atoms.

8. The compounds according to claim 1, wherein Het is N-alkoxymethylimidazole, wherein the alkoxy contains 1 to 4 carbon atoms.

9. The compounds according to claim 1, wherein Het¹ and Het² are individually selected from the group consisting of thiophene, furan, thiazole, oxazole, N-alkylpyrrole, imidazole, pyrazole and triazole, wherein any alkyl groups contain 1 to 4 carbon atoms.

10. The compounds according to claim 1, wherein Het¹ and Het² are individually selected from the group consisting of thiophen, furan, thiazole, oxazole, N-alkoxymethylpyrrole, imidazole, pyrazole, and triazole, wherein any alkoxy groups contain 1 to 4 carbon atoms.

11. The compounds according to claim 1, wherein A is a straight chain alkyl group of 1 to 6 carbon atoms.

12. The compounds according to claim 1, wherein A is an amidine, with an aliphatic chain, of the formula
$$-C_pH_{2p}-\overset{\text{NH}_2}{\underset{\text{NHX}}{\text{C}}}$$

wherein

p is 0 to 5 and X is selected from the group consisting of -H, -OH, -NH₂, -CH₃, -CH₂CH₃, and C₃H₇.

13. The compounds according to claim 1, wherein A is an amidine, wherein at least one nitrogen atom is a member of a five-membered heterocyclic structure.

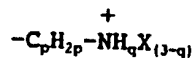
14. The compounds according to claim 1, wherein

A is a guanidine of formula $-C_pH_{2p}NH-C \begin{matrix} \nearrow NH \\ \searrow NHX \end{matrix}$

wherein p = 0 to 5 and X is selected from the group consisting of

-H, -OH, -NH₂, -CH₃, CH₂CH₃, and C₃H₇.

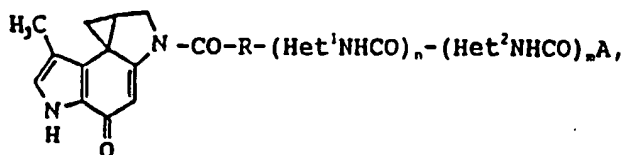
15. The compounds according to claim 1, wherein A is a quaternary, tertiary or secondary ammonium salt of the formula



wherein p = 0 to 5 and q is 0, 1, 2, 3, and X is an alkyl or alkenyl group of 1 to 3 carbon atoms.

16. The compounds according to claim 1, wherein A is a sulfonium salt of formula C_pH_{2p}-SXY, wherein p is 0 to 5 and X and Y are alkyl or alkenyl groups of 1 to 3 carbon atoms.

17. A pharmaceutical composition comprising a compound of the formula:



5 wherein,

Het¹ and Het² are individually selected from the group consisting of pyrrole, imidazole, triazole, thiophene, furan, thiazole, oxazole and pyrazole,

R is selected from the group consisting of a
10 valence bond; a C₁-C₆ alkyl; a C₂-C₆ alkenyl; a C₂-C₆ alkynyl; and an ortho, meta or para linked aromatic group,

A is selected from the group consisting of a C₁-C₆ alkyl group; an amidine or derivative thereof; a guanidine; a secondary, tertiary or quaternary ammonium
15 salt; and a sulfonium salt,

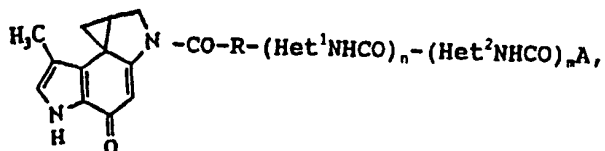
n is 0 to 3, and

m is 0 to 3,

in combination with a pharmaceutically acceptable carrier.

18. A method for alkylating specific DNA sequences in vivo, comprising administering to a patient in need of such alkylating, an amount of a compound of the formula:

5



wherein,

Het¹ and Het² are individually selected from the group consisting of pyrrole, imidazole, triazole, thiophene, furan, thiazole, oxazole and pyrazole,

10

R is selected from the group consisting of a valence bond; a C₁-C₆ alkyl; a C₂-C₆ alkenyl; a C₂-C₆ alkynyl; and an ortho, meta or para linked aromatic group,

A is selected from the group consisting of a C₁-C₆ alkyl group; an amidine or derivative thereof; a guanidine; a secondary, tertiary or quaternary ammonium salt; and a sulfonium salt,

15

n is 0 to 3, and

m is 0 to 3,

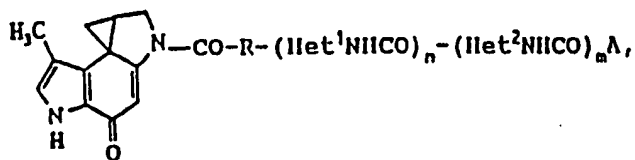
effective to alkylate specific DNA sequences.

20

19. The method according to claim 18, wherein said DNA sequences are oncogene DNA sequences.

20. A method of treating cancer comprising administering to a patient in need of such treatment, an amount of a compound of the formula:

5



wherein,

Het¹ and Het² are individually selected from the group consisting of pyrrole, imidazole, triazole, thiophene, furan, thiazole, oxazole and pyrazole,

10

R is selected from the group consisting of a valence bond; a C₁-C₆ alkyl; a C₂-C₆ alkenyl; a C₂-C₆ alkynyl; and an ortho, meta or para linked aromatic group,

A is selected from the group consisting of a C₁-C₆ alkyl group; an amidine or derivative thereof; a guanidine; a secondary, tertiary or quaternary ammonium salt; and a sulfonium salt,

15

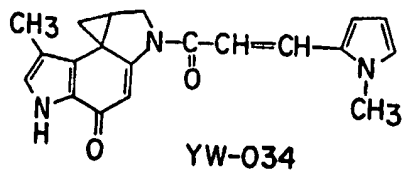
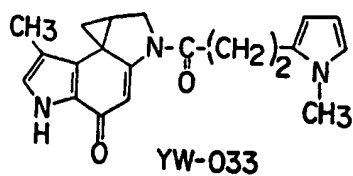
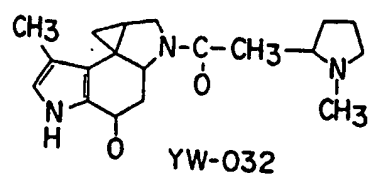
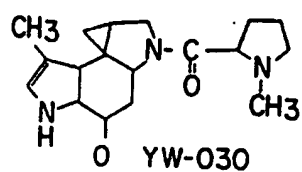
n is 0 to 3, and

m is 0 to 3,

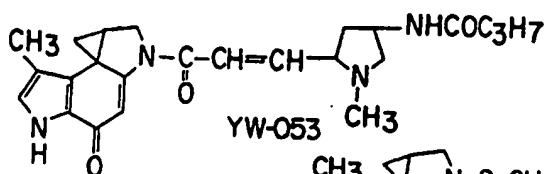
effective to treat said cancer.

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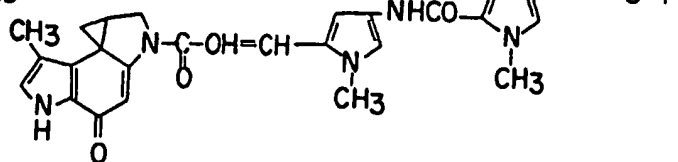
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*Fig. 1**Fig. 1A**Fig. 1B**Fig. 1A**Fig. 2**Fig. 2A**Fig. 2B**Fig. 2A*

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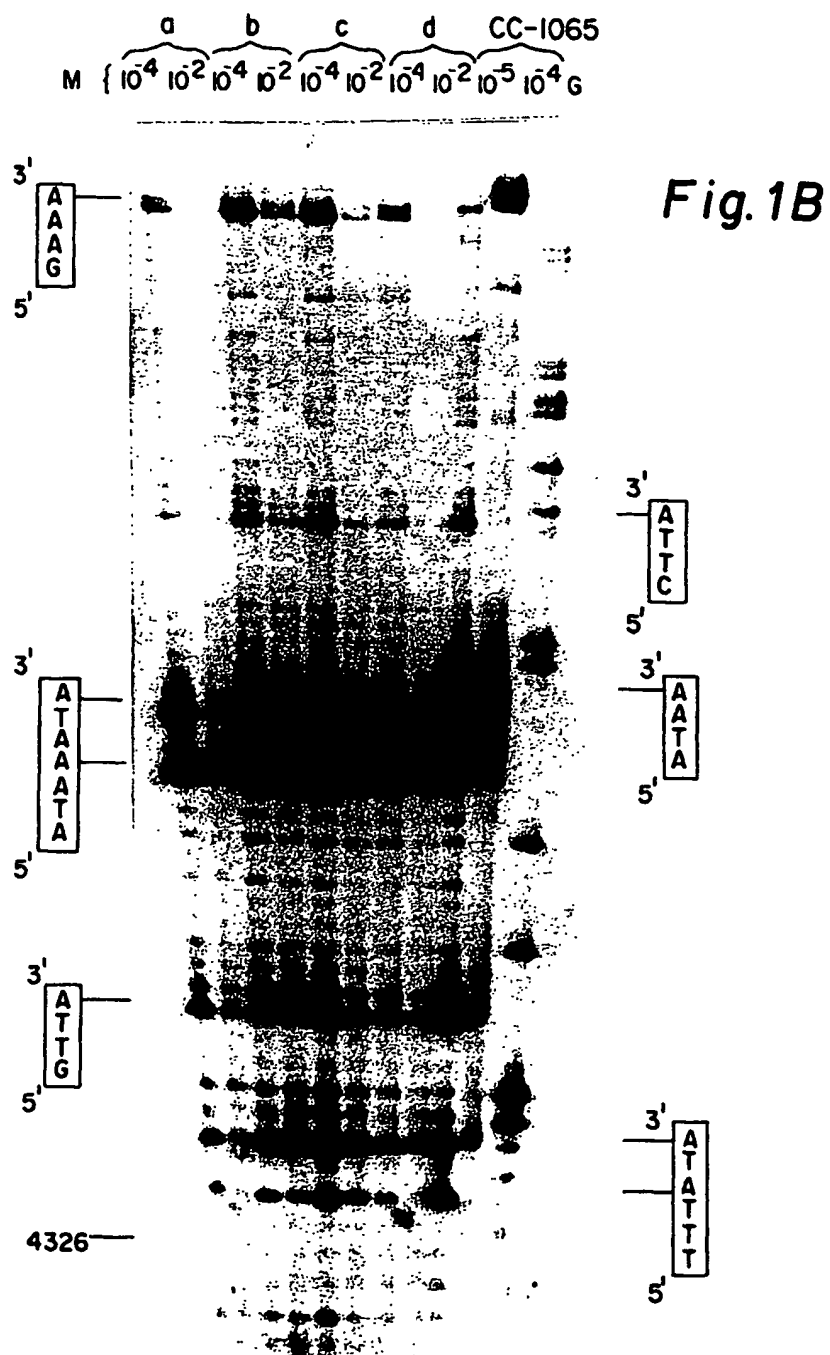


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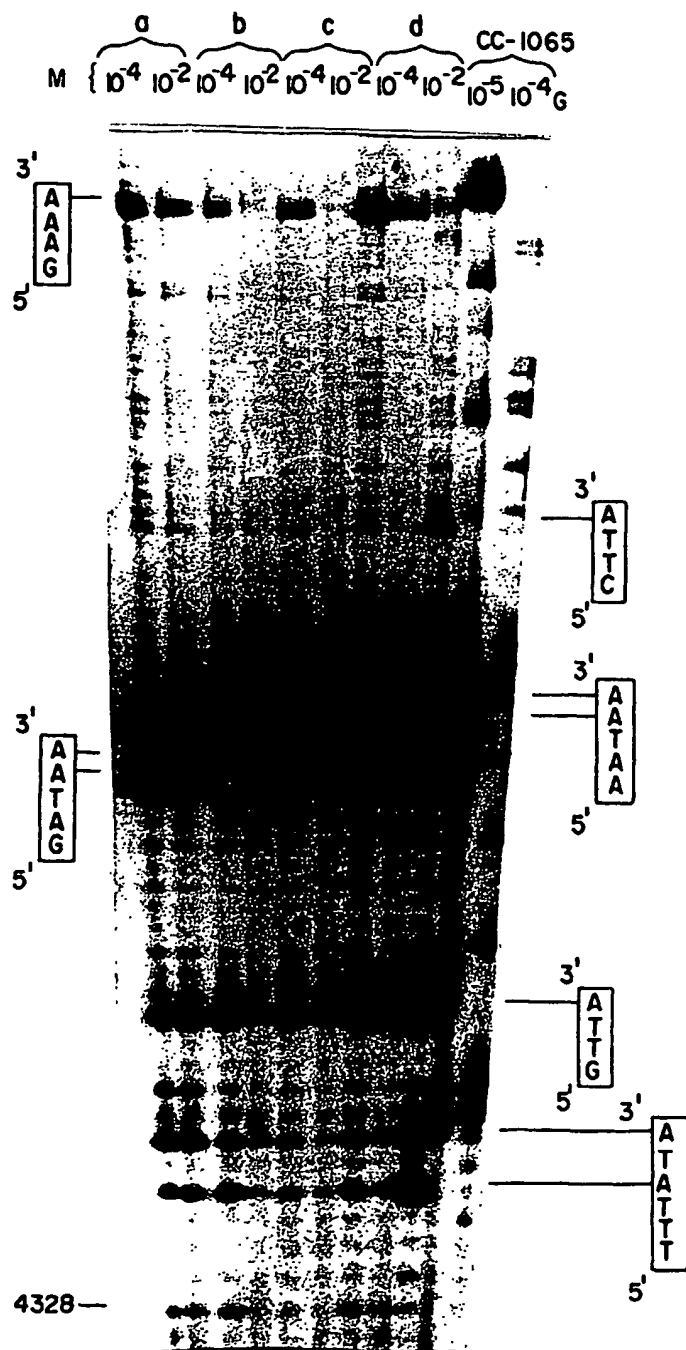
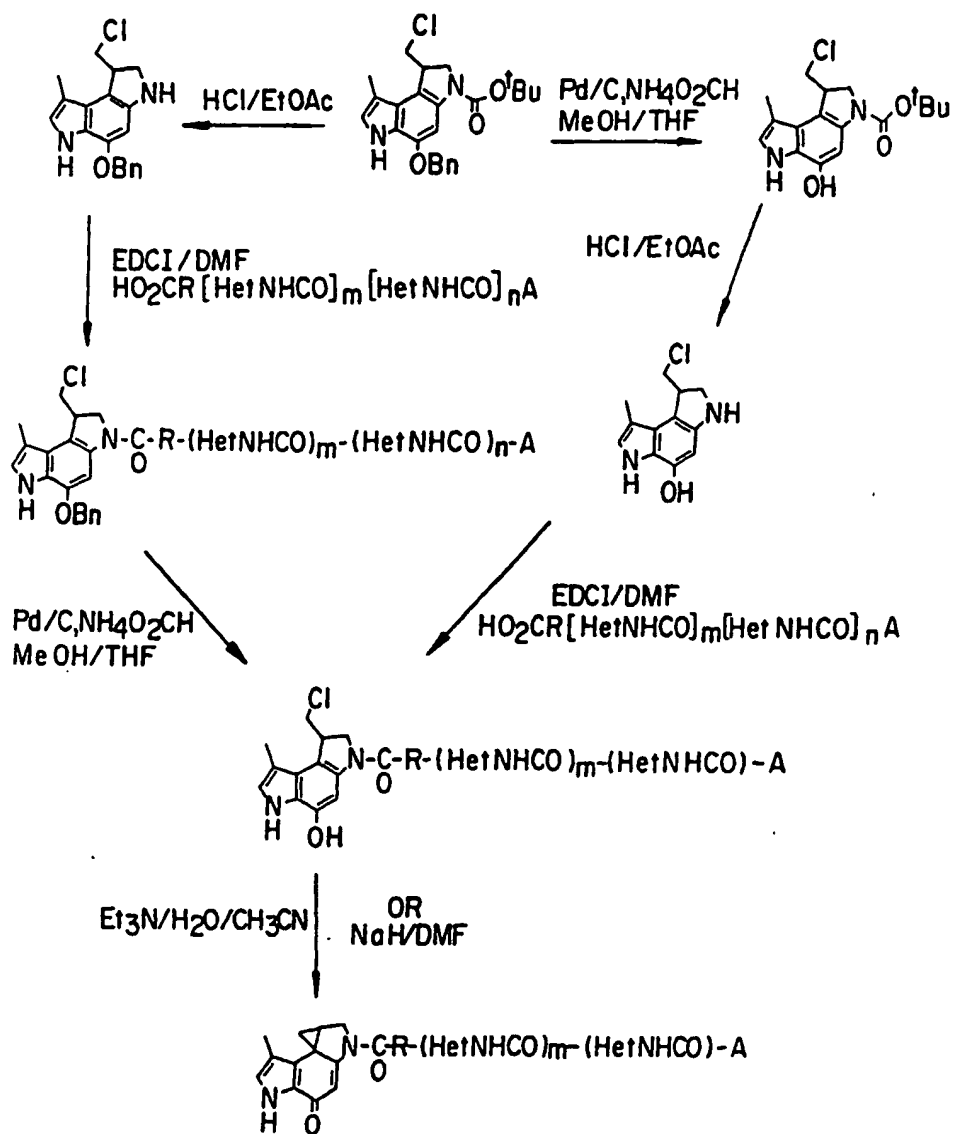


Fig. 2B

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Fig. 3



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